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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C12Q 1/68, G09F 3/00, D21H 21/46, G01N 33/00 — A1

(11) International Publication Number: WO 96/17954

(43) International Publication Date: 13 June 1996 (13.06.96)

(21) International Application Number: PCT/IB95/01144

(22) International Filing Date: 8 December 1995 (08.12.95)

(30) Priority Data:

944739	8 December 1994 (08.12.94)	NO
944740	8 December 1994 (08.12.94)	NO

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(81) Designated States: AL, AM, AT, AT (Utility model), AU, BB, BG, BR, BY, CA, CH, CN, CZ, CZ (Utility model), DE, DE (Utility model), DK, DK (Utility model), EE, EE (Utility model), ES, FI, FI (Utility model), GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK (Utility model), TJ, TM, TT, UA, UG, US, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG), ARIPO patent (KE, LS, MW, SD, SZ, UG).

Published

With international search report.

Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: CHEMICAL LABELLING OF OBJECTS

(57) Abstract

A method is provided for the labelling of objects such as industrial products, works of art, antiquities, securities, and environmental pollutants as well as of biological material such as living organisms and vira. The method comprises adding at least two chemical tags to the object. The information embedded in the first tag is not divulged to the public, comprises an informational content which can be amplified by means of molecular amplification (PCR), and which specifically identifies the identity and/or origin of the object. The second tag indicates the presence of the first label and is easily detectable. Also provided are the labelled objects and a method for determining the identity and/or origin of the labelled objects. It is preferred that the chemical tags comprise an informational content which is in the form of an alphanumeric code and are nucleic acid fragments such as DNA or RNA.

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Chemical labelling of objects

FIELD OF THE INVENTION

The present invention relates to the field of labelling and tracing objects such as industrial products, antiquities, works of art etc. as well as labelling and tracing biological material such as living organisms and vira. More specifically, the invention pertains to a method for chemical labelling of objects for later determination of their identity and/or origin, chemically labelled objects, and a method for the determination of identity and/or origin of such labelled objects.

GENERAL BACKGROUND

Labelling or tagging of physical objects in order to prevent or detect theft, counterfeit, forgery, infringement of copyright, unlawful or accidental pollution of the environment etc. requires that a number of criteria are met by the labelling method.

First of all, a label should exhibit a certain degree of specificity for the labelled object, since the value of the label as an identification marker will decrease with its progressive lack of specificity. The degree of specificity needed will of course be dependent on the scenario wherein the label is utilised (*i.e.* whether it has been used in order to specifically identify one single object or merely to identify the manufacturer of the product).

Secondly, the label should in many cases be resistant to removal from the object; it is for example desirable to label automobiles by another and more robust means than the serial number engraved in a part of the car, since such a serial number is fairly easy to remove or replace.

Finally, in many products a specific label should preferably be difficult to copy by an infringing third-party, in order to prevent unlicensed copying of the object in question.

In consequence, a number of methods for the labelling of objects have been suggested. Specifically, labelling of objects with nucleic acid fragments have been disclosed in a number of patent applications.

WO 90/14441 generally discloses the labelling and tracing of materials by use of nucleic acids. The tracing is preferably performed by the aid of amplification of the nucleic acids used for labelling.

EP-B-408,424 discloses the marking of valuable objects by applying a solution of a target nucleic acid, the solution having a chosen fluidity, to the objects and subsequently identifying the object if this is necessary by detecting the target nucleic acid.

WO 91/17265 pertains to the tracing of liquid and solid materials, especially oil, after the materials have been labelled with DNA molecules.

WO 94/04918 pertains to the marking of liquids with ≤ 1 ppm of particles comprising *i.a.* nucleic acid tags coupled thereto.

The use of nucleic acids as *the* preferred type of molecules for labelling involves a number of advantages. First of all, because of the existence of effective molecular amplification methods such as PCR, even trace amounts of nucleic acids can be detected and analyzed with respect to their sequence. Thus, the object which is labelled with the nucleic acid will be virtually unchanged by the addition of the label. Further, it is relatively simple to produce suitable nucleotide fragments by use of conventional DNA chemosynthesis.

Secondly, it is only possible to amplify such a nucleic acid fragment when sufficient information concerning the sequences of the 3' and the 5' ends of the molecule is available. If not, it is not possible to produce useful primers in order for the amplification reaction to occur, and it will therefore not be possible for a third-party to detect the presence of the label, let alone to decipher the message hidden in the sequence.

However, the labelling of products with nucleic acids (whether these are in the form of naturally occurring materials or in the form of synthetically produced fragments) involves the drawback of the need for a specific set of primers or hybridization probes in order to amplify and/or detect each specific label. In cases where the object which is suspected of being labelled with nucleic acids is found at a location where there is no direct access to the pertinent amplification primers, problems are encountered by the local authorities when attempting to establish e.g whether the object is stolen or whether it is at all labelled.

Thus, there is a definite need for methods of preparing labelled products which are easily identifiable *on location* as being labelled, and which at the same time conserve the advantages of using nucleic acids as labels in objects.

OBJECT OF THE INVENTION

It is therefore an object of the present invention to provide an improved method for the chemical labelling of objects which combines the advantages of chemical labelling by means of amplifiable molecules such as nucleic acids with means and measures which facilitate that labelled objects are readily identified.

BRIEF SUMMARY OF THE INVENTION

In order to overcome the drawbacks of the prior art methods, the present inventor has conceived a novel labelling scheme which combines the use of at least two sets of chemical tags comprising information which is contained in a chemical substance which can be subjected to molecular amplification.

In its broadest scope the present invention relates to a method for the chemical labelling of an object, the method comprising adding, to the object, at least two chemical tags, a first and a second,

- the first tag comprising an informational content 1) which is not divulged to the public, 2) which can be amplified by use of molecular amplification, and 3) the presence of which specifically establishes the identity and/or origin of the object,

- the second tag comprising an informational content 1) the presence of which indicates that the object is labelled with the first substance, and 2) which is easily detectable.

By the term "first chemical tag" (or just "first tag") is herein meant a substance or composition which comprises a part (carrying the informational content) which can be amplified by means of molecular amplification. Exemplary are compositions or substances comprising nucleic acid fragments which carry information and which can be amplified by e.g. PCR or other molecular amplification methods known in the art.

By the term "second chemical tag" is meant a substance or composition, the presence of which can be established without the need of expert knowledge or laboratory equipment and which further is used as an indicator of the presence of a first chemical tag.

Part of the first and second chemical tags may be a carrier or solid support such as latex beads, dextran or magnetic beads, polymeric substances or lipophilic compositions encapsulating the information bearing component.

It will be understood that the labels according to the present invention are normally in the form of two different chemical tags, one first and one second. It is, however, sometimes desirable to include more than one first chemical tag and/or more than one second chemical tag, since it then becomes possible to label the object with codes of varying or equal levels of security. It will for example be possible to label an object with several specifically identifying first chemical tags of different composition. Thereby, the task for a potential infringer can be made unsurmountable, since he will not know how many first tags to identify and copy before his infringing product will comprise a label identical to that of the first object.

The term "informational content" is herein meant to indicate a set of features of the structures of the chemical tag which can be interpreted as a message (typically the first chemical tag) or as an indication (typically the second tag). Exemplary is a chemical tag which comprises a nucleotide sequence which, when decoded, will provide a set of informations, preferably in the form of an alphanumeric code.

"Information bearing component" is the part of the chemical tag which can be directly decoded in order to obtain the informational content, for example one or more nucleic acid fragments forming part of the chemical tag. It will be understood that the information bearing component may comprise the total chemical tag (e.g. when the tag is in the form of one single free nucleic acid fragment) or it may form a part of the chemical tag to which it is optionally covalently or non-covalently bound. Further, the information bearing component may comprise several separate molecular entities, e.g. seve-

ral nucleic acid fragments, which together can be decoded to reveal the informational content.

By the term "object" is in the present specification and claims meant non-living materials or substances as well as biological and living material. The minimum requirement to an "object" as defined herein is simply that it is possible to provide the object with a label according to the present invention.

The term "adding to the object" means that the chemical tags are introduced on or in the object. The chemical tags can be suspended in the object, covalently or non-covalently bound to the object or to subunits of the object, applied as a film or a spray on the object, etc, depending of the nature and structure of the object to be labelled.

By the term "molecular amplification" is herein meant any amplification method which is capable of providing a large number of identical copies of a specific molecular entity from a single or only a few original copies of said specific molecular entity. At present the best known, and most widely used, molecular amplification method is the polymerase chain reaction (PCR) which is disclosed in detail in EP-B-200,362, EP-B-201,184, EP-A-258,017, EP-A-502,588, EP-A-502,589, EP-A-505,012, EP-A-509,612. The polymerase used according to these disclosures, the *Taq* polymerase may of course be exchanged by commercially available thermostable polymerases such as Vent® and AmpliTaq®.

By the expression "specifically establishing identity and/or origin" is meant that the informational content of the chosen first chemical tag differs sufficiently from that of other known chemical tags of the same nature in order for a positive identification of the object to be performed. This has two practical implications: 1) The first chemical tag should be constructed in such a way that the probability of finding an identical chemical tag in a different object or group of

objects is close to zero, and 2) if the first chemical tag is comprised of a molecule type which could theoretically be isolated from natural sources, it is necessary that the exact composition of the first chemical tag is such that the probability of finding the same molecule in nature is also close to zero. It will be understood, that since the first chemical tag will always be accompanied by the second chemical tag in an object labelled according to the present invention, the combined finding of these at least two tags in the same object will contribute to the minimization of the risk of finding the same label in another object or group of objects.

However, as a practical matter, one can imagine the situation wherein the first chemical tag is a DNA fragment of random sequence. If such a DNA fragment has a length of at least 30 nucleic acid residues, the probability of finding an identical consecutive stretch of nucleic acid residues in nature would be acceptably low. Any other first chemical tag exhibiting a similar or lower probability of "natural reproduction" will of course also be acceptable. However, in most practical embodiments of the invention, a nucleotide sequence utilised as the information bearing component will comprise at least 50 nucleotides, and more often at least 75 nucleotides. It is preferred that such a nucleic acid fragment will comprise at least a 100, such as at least 150, nucleotides.

By the term "easily detectable" is herein meant that the presence of the informational content of the second chemical tag can be demonstrated by relatively simple means which do not require the presence of laboratory equipment or any expert skill of the person performing the demonstration. Typically, the detection of the second chemical tag and its informational content can be provided by use of a simple test which can be performed "in the field" by a person unskilled in chemistry, physics, biochemistry, molecular biology etc.

Because of the presence of the easily detectable second chemical tag, it is fast and uncomplicated for the relevant

authorities to establish that an object is in fact labelled. As will be described in detail below, even in a number of cases wherein the absence of labelling is detected, this will immediately indicate that the object is stolen (e.g. cars which are routinely labelled according to the invention by the manufacturer).

Apart from the inventive process for labelling objects, the invention also pertains to objects which contain such labelling which can be provided by the labelling method of the invention. Thus, the invention also relates to an object comprising a chemical label which contains

- a first chemical tag comprising an informational content 1) which is not divulged to the public, 2) which can be amplified by use of molecular amplification, and 3) the presence of which specifically establishes the identity and/or origin of the object,

- a second tag comprising an informational content 1) the presence of which indicates that the object is labelled with the first substance, and 2) which is easily detectable.

Finally, the invention relates to a method for the determination of the identity and/or origin of an object, the method comprising subjecting an object (which is labelled by the method of the invention or is an object of the invention) to a determination which involves the steps of

- detecting the presence and/or extracting the informational content of the second tag, thereby obtaining an indication that the object is labelled with a first tag according to the invention, and subsequently

- detecting the presence and/or extracting the informational content of the first tag by methods involving molecular amplification of the first tag.

LEGENDS TO THE FIGURE

Figs. 1a and 1b shows one preferred way of identifying the information bearing component of a first (or optionally a second) chemical tag.

Fig. 1A schematically depicts a DNA template and four PCR primers covering the total length of the template, as well as the total outcome of the possible PCR reactions involving the template and the primers.

Fig. 1B shows schematically the outcome of a gel electrophoresis performed on the amplification products.

DETAILED DISCLOSURE OF THE INVENTION

Features of the first chemical tag

The informational content of the first chemical tag should according to the invention not be divulged to the public. This means that the exact nature of the information bearing component will not be known to the public. On the other hand, it is necessary that the manufacturer or owner of the object can demonstrate the informational content of the first chemical tag in a plausible manner, in order for the informational content to be effective as legal proof.

One possibility is that the legal person responsible for the labelling of the object deposits the following at a nationally or internationally accepted deposition institution: 1) The exact informational content, 2) an indication of the code used to prepare the information bearing component, 3) and a sample or precise description of the information bearing component and optionally of the total first chemical tag. The deposited matter will be assigned a deposition number and a date of deposit, and at a later date the deposited matter can be extracted upon request from the depositor.

It will be understood that the specificity of the informational content of the first label will depend on the speci-

fic scenario wherein the label is used. In the cases wherein one single object is sought protected (such as an important work of art) the label will normally specify precisely both the identity of the object and its origin, *i.e.* the informational content of the first tag is assigned specifically to one single object. On the other hand, in the case of mass-produced products where the risk is that of illicit copying of the product rather than theft thereof, the informational content is assigned specifically to a group of objects, namely all the objects produced by a certain manufacturer.

In a preferred embodiment of the invention, the informational content of the information bearing component in the first tag is in the form of an alphanumeric code; this is especially interesting when using a linear molecule such as a nucleic acid as the information bearing component in the first and/or second chemical tag. Such an alphanumeric code can be envisaged in an infinite number of ways.

By the term "alphanumeric code" is herein meant the direct coupling between specific molecular configurations (e.g. nucleotide sequences) and alphanumeric characters. In the following, alphanumeric codes based on nucleotide sequences is discussed:

The "code unit" is the number of nucleotides which forms one single character in a chosen alphanumeric nucleotide code, *i.e.* in a chosen alphanumeric code, two occurrences of the same code unit will (if they are in the same reading frame) indicate the same alphanumeric character.

By the term "reading frame" is in this context meant a consecutive stretch of nucleotide sequences each having the length of one code unit. Thus, two code units are in the same reading frame when they make up two such nucleotide sequences in a reading frame.

The possible number of different characters in such an alphanumeric code depends on two numbers, namely the number of different nucleotides used in the nucleic acid fragment (usually 4) and the number of nucleotides used to form each character. More specifically, the maximum number of characters in the alphanumeric code will vary according to the formula I:

$$N \leq Z^n \qquad (I)$$

wherein N is the number of characters in the chosen code, Z is the number of different nucleotides used in the nucleic acid fragment, and n is the number of nucleotides forming each character. For instance, when only using the four naturally occurring nucleotides A, T, G and C, the number of alphanumeric characters in the code will be at most 64 when using triplets of nucleotides for each character, but 256 when using quadruplets for each character; in other words, an alphanumeric code based on 4 nucleotides and a code unit of 4 nucleotides can carry one byte of information.

In the following tables 1A and 1B are given an example of an alphanumeric code wherein n=3 (a triplet code) and the number of nucleotides, Z, is four (A, T, G and C); Table 1A shows the possible triplets and Table 1B shows one exemplary code which can be assigned to these triplets:

TABLE 1A

	T	C	A	G	
T	TTT	TCT	TAT	TGT	T
T	TTC	TCC	TAC	TGC	C
T	TTA	TCA	TAA	TGA	A
T	TTG	TCG	TAG	TGG	G
C	CTT	CCT	CAT	CGT	T
C	CTC	CCC	CAC	CGC	C
C	CTA	CCA	CAA	CGA	A
C	CTG	CCG	CAG	CGG	G
A	ATT	ACT	AAT	AGT	T
A	ATC	ACC	AAC	AGC	C
A	ATA	ACA	AAA	AGA	A
A	ATG	ACG	AAG	AGG	G
G	GTT	GCT	GAT	GGT	T
G	GTC	GCC	GAC	GGC	C
G	GTA	GCA	GAA	GGA	A
G	GTG	GCG	GAG	GGG	G

TABLE 1B

	T	C	A	G	
T	0	1	2	3	T
T	4	5	6	7	C
T	8	9	:	;	A
T	<	=	>	?	G
C	@	A	B	C	T
C	D	E	F	G	C
C	H	I	J	K	A
C	L	M	N	O	G
A	P	Q	R	S	T
A	T	U	V	W	C
A	X	Y	Z	Æ	A
A	Ö	Å	^	-	G
G	.	*			T
G					C
G					A
G					G

Since the use of the above table 1B would result in nucleotide fragments with a relatively low content of guanosine (G), the blank fields in the above table can e.g. be filled out with characters in order to ensure an equal balancing of the occurrence of all possible triplets (the characters "E" and "S" could e.g. be coded by more than one triplet in order to compensate for the high frequency of these letters in the british language).

The advantage of using an alphanumeric code is primarily that the interpretation of the code, once the key to the code is

known, is straightforward and can be comprehended by most people. For instance, in a jury trial, there would be no need of expert witnesses to interpret technical evidence, once the basic code matrix has been revealed.

It will be understood that any combination of characters can be introduced in a table like the above in order to generate a new code. However, it will be desirable that a relatively restricted number of standard code tables are generated, since this will improve the liability in e.g. a court room when the interpretation of the informational content is disclosed. This will also involve the advantage that merely a code number would have to be deposited at the above-outlined deposition institution.

The main candidates for molecules which are susceptible to molecular amplification are nucleic acids, and it is thus preferred that the first tag (and optionally the second tag) comprises a nucleic acid fragment, such as a DNA or RNA fragment. As discussed herein, this allows for the coding of the label in terms of a message which is embedded in the sequence of the nucleic acid fragment. Depending on the coding matrix chosen, the code can involve an indefinite number of characters. Normally, a nucleotide sequence carrying a code will exclusively contain the four ribonucleotides A, G, C, and U (in the case of RNA) or the four deoxyribonucleotides dA, dG, dC, and T (in the case of DNA), but in order to either 1) increase the number of characters in the code without extending the length of the code unit, 2) to alter the solubility of the nucleic acid fragment, or 3) to protect the nucleic acid fragment against enzymatic degradation, nucleotides different from A, dA, G, dG, C, dC, U, and T may be used; these different nucleotides may be used as substitutes for the naturally occurring bases. The use of modified, non-naturally occurring or rare nucleotides also involves the advantage that breaking of the code (by sequencing) becomes increasingly difficult for an infringing third-party.

Especially preferred modified nucleotides to be used in the chemical tags of the invention (when these comprise nucleic acids) are selected from the group consisting of

4-acetylcytidine,
5-(carboxyhydroxymethyl)uridine,
2'-O-methylcytidine,
5-carboxymethylaminomethyl-2-thiouridine,
5-carboxymethylaminomethyluridine,
dihydrouridine,
2'-O-methylpseudouridine,
beta,D-galactosylqueuosine,
2'-O-methylguanosine,
inosine,
N6-isopentenyladenosine,
1-methyladenosine,
1-methylpseudouridine,
1-methylguanosine,
1-methylinosine,
2,2-dimethylguanosine,
2-methyladenosine,
2-methylguanosine,
3-methylcytidine,
5-methylcytidine,
N6-methyladenosine,
7-methylguanosine,
5-methylaminomethyluridine,
5-methoxyaminomethyl-2-thiouridine,
beta,D-mannosylqueuosine,
5-methoxycarbonylmethyl-2-thiouridine,
5-methoxycarbonylmethyluridine,
5-methoxyuridine,
2-methylthio-N6-isopentenyladenosine,
N-((9-beta-D-ribofuranosyl-2-methylthiopurine-6-yl)carbamoyl)threonine,
N-((9-beta-D-ribofuranosylpurine-6-yl)N-methylcarbamoyl)threonine,
uridine-5-oxyacetic acid-methylester,

uridine-5-oxyacetic aid,
wybotoxosine,
pseudouridine,
queuosine,
2-thiocytidine,
5-methyl-2-thiouridine,
2-thiouridine,
4-thiouridine,
5-methyluridine,
N-((9-beta-D-ribofuranosylpurine-6-yl)-carbamoyl)threonine,
2'-O-methyl-5-methyluridine,
2'-O-methyluridine,
wybutosine, and
3-(3-amino-3-carboxypropyl)uridine.

It is of course also possible to superpose an encryption on the alphanumeric coding. In such a case, the direct interpretation of the decoded chemical tag will, however, give no direct meaning and the informational content must be retrieved by deciphering by means of the inverse encryption key.

Non-alphanumeric coding

Another possibility would be to assign a non-alphanumeric code to a chemical tag used in the invention. The requirements for such a code will be identical to that of an alphanumeric, *i.e.* it must specifically and/or unambiguously identify the object and/or its origin. However, the number of degrees of freedom increases when a non-alphanumeric code is used, since every unique chemical tag may be assigned a code which can have any possible length. It is however preferred to use the alphanumeric coding system of the first tag for the reasons set forth above.

Under all circumstances, when using nucleic acid fragments as the information bearing component, it is preferred that these are produced by conventional automated nucleotide synthesis

(since their sequence can be precisely predetermined), but the fragments may also be produced biologically and isolated from such sources.

Stability requirements of the first chemical tag

Depending on the character of the object to be labelled, the stability of the labelling substances may be of lesser or greater concern.

Labels which are to be used in objects which are made of materials which are likely to be recycled: Such labels should preferably be produced so as to be destroyed when going through the recycling process.

One example is labels which are incorporated into plastic materials which are systematically recycled for use in the production of new plastic materials. Such labels will need to be rendered unstable under the conditions which allow the recycling of the object. One attractive approach is to use RNA as part of the first chemical tag, since this molecule is highly susceptible to environmentally abundant RNases. The requirement would then be that the RNA containing tag is protected from exposure to RNase when in use, e.g. by means of encapsulation (for example in polymers or in micelles), methylation of the nucleotides, formulation of the RNA fragments together with stabilising aliphatic proteins, provision of double stranded RNA fragments etc.

Another example is the labelling of alcoholic beverages, perfumes and other liquids. Tags containing RNA could be added to the liquid on the location of manufacture under contamination-free conditions. It will of course only be feasible to amplify RNA from an unopened bottle, but if a stock of counterfeit goods have been confiscated, the demonstration of the absence of such a label in unopened bottles indicate that the content of the bottles has not been manufactured by the lawful manufacturer.

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Labels which are to be used in objects where their destruction is an undesired possibility: When using nucleic acid fragment labels in oil products in order to trace the origin of oil pollution at sea it is highly likely that attempts will be made to remove the label prior to illegal dumping of oil. Therefore, the label should be constructed in such a way that the first and second tags are highly resistant to degradation, e.g. chemical or enzymatic degradation. This can be achieved by methylation of ribonucleotides/deoxyribonucleotides, encapsulation (for example in polymers or in micelles), stabilising of the nucleic acids by formulation together with aliphatic proteins, use of double stranded nucleic acid fragments, etc, or combinations thereof.

Formulation of the chemical tags

As discussed in detail below, it is sometimes important that the first chemical tag is formulated in such a way that it is compatible with the labelled object or comprises a moiety which ensures compatibility with the object. This can be achieved in a number of ways; normally the problem will be that of ensuring an adequate *solubility* of the tag in a non-aqueous liquid. For instance, the above-mentioned moiety of the tag can be covalently or non-covalently attached to the remainder of the tag and the moiety can then in itself be compatible with the object or be coupled to a third substance which is compatible with the object.

Alternatively the tags can be formulated together with a micelle forming substance or a detergent, thereby enhancing solubility in the object or in the formulation applied on the object.

However, when using nucleic acids as the information bearing component in the first (and optionally in the second) chemical tag, it will normally be of minor importance how the tag is formulated since the very low concentrations of the nucleic acids will not reveal their presence, regardless of

whether they are soluble or not in the medium where they are suspended.

In certain embodiments, however, the question of formulation *is* of importance, namely when the tags are added to oils or petrochemical products which are liable to get in continuous contact with large quantities of aqueous material (e.g. labels present in crude oil which has leaked from a oil transport at sea) which could potentially wash the tags out of the product. Under such circumstances it must be ensured that the nucleic acids are formulated in order for them to favour solubilisation in hydrocarbons instead of an aqueous phase so as to prevent wash-out of the hydrocarbon phase of the nucleic acid.

Such enhanced solubilisation in hydrocarbons can be achieved in a number of ways: Parts or all of the nucleic acid may be methylated, the nucleotides could be coupled to biotin or hydrophobic haptens such as fluorescein, dinitrophenol and tri-iodothyronine, or sulphonucleotides containing thiophosphates could be used and incorporated in the nucleic acid and subsequently derivatized with thiol-specific modifying agents such as iodoethanol. Alternatively the nucleic acids may be covalently linked to hydrophobic beads (e.g. magnetic) designed to be soluble in hydrocarbons and not in an aqueous phase.

The above-mentioned types of formulations of nucleic acids (especially of DNA) are discussed in detail in WO 91/17265 and in WO 94/04918.

Nature of the second chemical tag

The second chemical tag should satisfy the same general criteria as the first chemical tag, *i.e.* it should exhibit a stability similar to that of the first chemical tag, so as to ensure that the labelled object will preserve the

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informational content of both tags to substantially the same degree.

According to the invention it is also necessary that the second chemical tag is easily detectable, meaning that it does not require expert skill or laboratory conditions to detect the second chemical tag.

Finally, the second chemical tag should preferably be present in the object in such a low concentration or amount that the presence of the second chemical tag does not interfere with the features of the object itself.

Thus, the second chemical tag can be selected from the group consisting of a nucleotide fragment, a rare metal, a substance capable of forming micelles, a stain (optionally a fluorescent dye), a specific binding partner such as an antigen capable of binding to an antibody coupled to a marker designed for easy detection, an antibody capable of binding to an antigen coupled to a marker designed for easy detection, and an enzyme capable of catalyzing an easily detectable chemical reaction.

It is, however, preferred that also the second chemical tag comprises an informational content which can be amplified by means of molecular amplification (cf. the discussion below pertaining to the detection of the labelled objects of the invention), because this allows for the use of only trace amounts of the second chemical tag thereby keeping down costs for the labelling procedure and avoiding interference with the features of the labelled object.

In light of this, each and every aspect of the invention which pertains to amplifiable first chemical tags of the invention pertains *mutatis mutandis* to the second chemical tags of the invention when the informational content of these can be amplified by means of molecular amplification. Thus, the informational content of the second chemical tag may be

in the form of an alphanumeric code, and it may be detectable as a result of the molecular amplification or of a combination of the molecular amplification and sequence analysis, etc.

Features relating to the nature of the object

It will be understood from the discussion herein that the labelling method according to the present invention is suitable for the labelling of a non-living article, product or composition. In these embodiments of the invention the problems relating to formulation of the chemical tags are relatively easily solved (cf. however the above discussion of oils and petrochemical products), simply because the chemical tag may be added in such a low concentration that it solubility in the formulation is normally of minor importance.

The non-living article, product or composition can therefore be any product or artifact it is desired to label, since the label may be introduced so as to form part of the object but also as a simple external marking, cf. for example the disclosure in EP-B-408,424 which relates to the labelling of valuable objects with nucleic acids.

The non-living materials or substances include an industrial product, a work of art, an antiquity, an environmental pollutant, air pollutants, oils and petrochemical products, aromatic and aliphatic compounds, explosives, foods and feeds, medicaments, inks, paper goods including securities such as bank notes and bonds.

However, equally important aspects of the invention relate to the labelling of biological material, *i.e.* living organisms and vira. Living organisms could be labelled for a number of reasons parallel to those justifying labelling of non-living material. Owners of microorganisms, cell lines or transgenic animals which are used in industrial processes would benefit from the labelling of the microorganisms, since it would be

possible to detect illicit use of said microorganisms by third-parties. In such scenarios it is preferred that the labelling according to the present invention is introduced as DNA or RNA (dependent on the type of organism or virus) in the genome or (less preferred due to the possible loss/dissemination of the label) in a plasmid of the living organism and in such a way that the label is passed on to the offspring of the first generation of labelled organisms (*i.e.* in higher organisms the label should be introduced in the germ cells). The introduction of the labels can be performed according to standard methods in the field of genetic engineering which are well known to the skilled person, cf. e.g. Sambrook J, Fritsch E F, and Maniatis T (1989), Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y. The introduction of the label in the virus or organism must, however, be performed so as to avoid any substantial interference with the phenotype, *i.e.* the introduction of the label should not lead to any altering in the expression pattern normally exhibited by the living organism.

Alternatively, the labelled living organism may be one individual being which is very valuable (a racing animal such as a horse or a breeding animal). In such a case the label should not be introduced into the germ line cells, but rather into a chosen subset of somatic cells which can be tested later on. In this embodiment, the label can also be introduced in the form of a physiologically acceptable implant (e.g. a PVC implant).

It is preferred that the labelled organism is selected from the group consisting of a prokaryotic organism which can be a bacterium, a blue green algae, or an intracellular parasite such as a mycoplasma; an eukaryotic organism selected from the group consisting of a yeast, a fungus, a protozoa, an algae, and a multicellular plant or animal; and cells/tissues derived from a multicellular plant or animal.

Detection of the informational content in labelled objects according to the invention

The detection of the second chemical tag should, according to the invention be the first step in the total detection procedure. The means and measures for detection of the second chemical tag (especially its informational content) will of course vary according to the nature of the second chemical tag, but the detection procedures are well-known in the art. In cases where the information bearing component is a ligand such as an antigen or an antibody, the detection system involves binding of the ligand to a specific binding partner (e.g. an antibody reacting with the antigen and *vice versa*; in such cases the detection system could be any immunological assay such as an RIA [radio immune assay], an EIA [enzymatic immune assay] such as an ELISA etc). In cases where the information bearing component of the second chemical tag is a rare metal, the detection can involve spectroscopy (e.g. flame spectroscopy). When the information bearing component of the second chemical tag is an enzyme, the detection phase could involve the demonstration of catalyzation of the pertinent reaction by the enzyme, etc.

It is preferred that the informational content of the first (and optionally of the second) tag is detectable as a result of the molecular amplification or of a combination of the molecular amplification and sequence analysis. This has the advantage that the label can be present in the object in such small concentrations that it will be virtually, if not totally, impossible to detect the presence of the label by any other means than the use of a method involving the molecular amplification.

As outlined above, the second chemical tag may also share the feature of the first chemical tag that the informational content can be amplified by use of molecular amplification. This is a preferred embodiment of the invention, since the easy detection of such a second tag can be accomplished by

way of relatively simple means. First of all, the second chemical tag should be easy to amplify for the relevant authority, *i.e.* the primers necessary for a positive demonstration of the presence of the informational content of the second tag should be available to the relevant authority, optionally also to the public. This can be accomplished by establishing a set of standards (or even one unique standard) which are nationally or internationally recognized as indicators of the presence of a second chemical tag. Such a standard could e.g. be a DNA fragment of approximately 100 nucleotide residues which is used for all products labelled according to the invention.

In order for the local authority to determine whether an object is in fact labelled according to the present invention, all what is needed is the relevant set of primers (which would be identical all over the world) and the necessary means for performing an amplification analysis. Since it is not necessary to totally avoid contamination from other sources in this step of the detection (since the result is only intended to indicate that the product is labelled according to the invention), a PCR reaction performed on a primitive (optionally portable) thermocycler would suffice in cases where the second chemical tag comprises DNA as the information bearing component.

Under special circumstances where a nucleic acid fragment acting as the information bearing component in a first or a second chemical tag has been rendered lipophilic (as discussed above in the section pertaining to formulation of the tags) it will be necessary to transfer the tag to an aqueous environment in order to complete a molecular amplification reaction and a subsequent label determination. Methods for achieving this goal is *i.a.* discussed in detail in WO 91/17265.

The determination of the informational content of the first tag can be accomplished only after the provision of the

relevant amplification primers and optionally of relevant sequencing primers.

In one preferred embodiment, the set of amplification primers relating to the information bearing component of the first chemical tag is constructed in such a way that they together cover the complete sequence of the information bearing component (cf. Fig. 1). In Fig 1a. is shown the relationship between the four primers P1 (20 nucleotides), P2 (29 nucleotides), P3 (20 nucleotides) and P4 (30 nucleotides) and a template DNA fragment (forming the information bearing component) of 99 nucleotides. The possible PCR reactions involving the use of the four primers will result in four different PCR fragments of 99, 79, 69 and 49 nucleotides, respectively (cf. Fig. 1B which shows the outcome of an agarose gel electrophoresis performed on the amplification products). If the PCR reaction is performed under strict amplification conditions, the homology between primers and template strings must be close to 100% in order for the amplification to occur and therefore the identity of the template string is established with high security. This principle can of course be envisaged with other templates and primers of different lengths and compositions.

However, if need be, the result can of course be confirmed by sequence analysis, e.g. by use of Sanger's dideoxy chain termination technique (Sanger *et al.* (1977), Proc. Natl. Acad. Sci. USA, **74**, 5463-5467) or by means of any other relevant sequencing technique.

Relation between the first and the second chemical tag

Normally the first and second chemical tags will be in the form of at least two separate chemical entities (*i.e.* in the form of at least two different and separate molecules) but in certain embodiments they may be part of the same chemical entity, e.g. of the same molecule. This embodiment of the invention is especially interesting when the second tag is in

the form of nucleotide fragments which flank the nucleotide fragment comprised in the first tag, since nucleotide stretches of the second tag may 1) serve as templates for primers in the amplification reaction amplifying the informational content of the first chemical tag and 2) serve as templates for primers in a possible subsequent sequencing reaction.

This embodiment of the invention can also be envisaged in the following way: The parts of the template strand which is complementary to the primers could be made of PNA (protein nucleic acids, cf. Nielsen P. E. *et al.*, 1991, Science **254**, pp. 1497-1500, which is coupled to either RNA or DNA which makes up the rest of the template strand. This approach would open up for increased fine tuning of the initial annealing between template and primers, since PNA has been demonstrated to exhibit superiorly dynamic hybridization characteristics compared to those of DNA and RNA.

Other embodiments

A very interesting embodiment of the invention utilises the differences in stability of the labels according to the invention. The label may according to this aspect of the invention contain at least one first tag which is subject to modification and/or loss of at least a part of its informational content as a consequence of its exposure to a physical or chemical condition. It is for example possible to construct labels containing a predetermined concentration of first tags. Samples of such labels are then subjected to well-defined exposures of the relevant physical or chemical condition. From these exposure experiments it is possible to establish a correlation between a) the (relative) amount of remaining first label which can be amplified and b) the exposure of the physical or chemical condition. Thereafter, it will be possible to use the correlation "backwards" in order to determine the exposure on the basis of the (relative) amount of remaining first tag in a sample which has

been subjected to an unknown exposure of the physical or chemical condition. This "assay" can be enhanced by using more than one first tag, e.g. nucleic acid fragments of different length or structure, whereby it is achieved that more than one correlation is performed.

It will further be possible to introduce "points of instability" in DNA fragments constituting the information bearing component and thereby to modulate the stability of the chosen chemical tag. This can be done by introducing one or several ribonucleotides in the sequence instead of deoxyribonucleotides, thereby defining points or stretches in the nucleic acid which will be less stable than the rest of the molecule.

The specific physical conditions may be radiation or high temperature and the chemical condition may be treatment with denaturing agents or enzymes. Therefore, by use of the invention it will be possible to establish whether e.g. a sterilisation procedure has been effective or whether objects have been subjected to an undesired exposure of e.g. radiation; in both cases the absence or significantly decreased concentration of the label will indicate that the exposure has occurred, and the degree of exposure can also be determined from the correlation.

EXAMPLES

In the following, a number of examples are given wherein the labelling method of the invention have proved useful or is considered advantageous over the presently available identification systems. In these examples, the nature of the second chemical label is not discussed in detail, but any of the labelling schemes mentioned above in the present specification can be employed.

Further, in the following examples "a chemical tag" generally denotes oligonucleotides, preferably DNA-oligonucleotides produced by conventional automated DNA chemosynthesis. The

sequence of the chemical tag is defined according to a predefined matrix, cf. the above discussion and tables 1A and 1B, allowing a pertinent and user-chosen alpha-numeric message to be introduced. The size of the oligonucleotides can vary substantially, but is normally around 100 nucleotides. In the following examples, chemical tags comprising DNA are exemplified, but it will be understood that the invention is in no way confined to this embodiment, cf. the above discussion of the nature of the first and second chemical tag.

The method for demonstration of DNA tags in objects (which may both be biological material and non-biological products) is the polymerase chain reaction (PCR), or any biochemically related enzymatic method for the amplification of nucleotides.

Detection and deciphering of the encoded message is carried out via the use of a suitable number of PCR cycles and reagents. In many of the following examples, the following outline conditions are used: 0.3 μM of each primer, 1.5 u Taq pol, 1,5 mM MgCl2, 0,2 mM dNTP in 0,050 ml reaction volume. PCR: Denaturation at about 94°C for 1 min., annealing at the relevant annealing temperature (ranging from 45-70°C, but typically 58°C for 1 min if nothing else is mentioned), extension at about 72°C for 1 min. The result is thereafter visualized by gel electrophoresis, staining of DNA with ethidiumbromide and photography using 301 nm UV light.

Alternatively, a combination of PCR and DNA sequence analysis is carried out to read the alphanumeric information of the tag; the sequence analysis may be performed according to the well-known dideoxy chain termination method of Sanger *et al.*

LABELLING OF LIVING MATERIAL

The method of the invention offers an invisible, nontoxic, safe chemical first tag which may (when labelling germ cells) or may not (when labelling somatic cells) be transmitted to

the next generation and which can be displayed as ID alpha-numerical characters by means of a laboratory analysis. The direct display of an owners chosen ID information is of utmost importance as evidence before e.g. a court or a jury.

LABELLING OF SOMATIC CELLS IN ANIMALS

It is contemplated that various animals can be advantageously labelled according to the invention. Illicit use of many animals is at present difficult to control and prevent.

One example is the ID labelling of race horses. A non-transmittable alpha-numeric ID-tag (which do not have any biological activity) would make it possible to unambiguously identify one single individual horse.

Other examples of animals include: Artificially Inseminated bulls (AI bulls), valuable pet animals (dogs, cats), experimental animal individuals (including transgenic mouse strains) and any other animal it would be of interest to unambiguously identify for fiscal, environmental or other reasons.

The DNA tags are introduced into the animal genome by DNA containing functional genes which can, when injected into muscle tissue, be expressed in a transient fashion (only for a limited period of time). This situation does, as opposed to the general methods of genetic engineering of the animal germ line, not result in a DNA tag which is transmitted to future generations. Only somatic cells are treated, either via injection or with a biolistic approach (gene gun); suitable vectors include a viral carrier using CAT plasmids (Gorman C. M. *et al.*, 1982, Mol. Cell. Biol. **2**, 1044, and Edlund T. *et al.*, 1985, Science **230**, 912) or poly(dI-dC):poly(dI-dC) as used by Singh *et al.* (Singh H. *et al.*, 1986, Nature **319**, 154). In order to obtain a stable chemical tag according to the invention, the somatic cells must integrate the DNA tag into their chromosomes. A recently described method for

facilitated transport of DNA into the nuclei of the recipient cells (Alestrôm *et al.* 1995, Transgenic Research, in press) will enhance the efficiency of the method of labelling of somatic cells according to the invention.

The detection is performed by use of a 30 cycle PCR as outlined above.

LABELLING OF GERM CELLS IN ANIMALS

One example is the labelling of fish strains used in aquaculture. Such fish strains are normally not protected by intellectual property rights and can thus easily be unlawfully exploited by others. A genetically transmitted alphanumeric ID-tag (which do not have any biological activity and do not affect or impair with the genetic traits of the fish) would make it possible to demonstrate that competitors are using the same strains of farmed fish.

An additional benefit would be of environmental nature. If escaped farm fish can be linked to the responsible farmer/fishfarming industry, legal matters of ecological and economical consequences can be resolved. Especially in the case that transgenic fish (or other aquatic animals) will be accepted in future commercial aquaculture, the public demand for an owner tag might be a prerequisite.

The DNA tags are introduced into the genome by general methods of genetic engineering (cf. Sambrook J, Fritsch E F, and Maniatis T (1989), Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.) and will be integrated into the chromosomes of the animal germ line in order to, as a minor addition to the genome (preferably having no biological activity), be transmitted to all future generations.

Other examples of animals include: AI bulls, valuable pet animals (dogs, cats), experimental animal strains (including

transgenic mouse strains) and any other animal it would be of interest to unambiguously identify for fiscal, environmental or other reasons.

The detection is performed by use of a 30 cycle PCR as outlined above.

LABELLING OF MICROORGANISMS IN FOOD INDUSTRIES

Microorganisms in food industries belong to a large group of valuable microbe strains with unique traits being more or less well-defined genetically. Illicit use of such microorganisms is at present difficult to control and prevent.

One example is the lactic acid and propionic acid bacterial strains which provides the Norwegian Jarlsberg cheese with its typical flavour. These bacterial strains are not covered by intellectual property rights and can thus easily be misused by third-parties. A genetically transmitted alphanumeric ID-tag (which do not affect or impair with the traits of the bacterium) would make it possible to show whether competitors are using the specific Jarlsberg strains.

The DNA tags are introduced into the bacterial genome by means of well-known general methods of genetic engineering (Sambrook J, Fritsch E F, and Maniatis T (1989), Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.) and will be integrated into the chromosome of the microorganism in order to, as a minor addition to the genome (which do not have any biological activity and do not affect or impair the genetic traits of the bacterium), be transmitted to all future generations.

The detection is performed by use of a 30 cycle PCR as outlined above.

LABELLING OF NON-LIVING OBJECTS

The method of the invention offers an invisible, nontoxic, safe chemical label which is displayed as ID alpha-numerical characters by means of a laboratory analysis. The direct display of an authority's, or owners, ID information will be of greatest importance as proof in for example a court or a jury.

The scale of synthesis of suitable DNA labels is at present adapted for analytical purposes, but can easily be scaled up for certain applications. The size of the oligonucleotides are typically around 100 nucleotide residues, but can vary substantially in some cases. The "small" scale (0.2 μmol) synthesis gives typically approximately 10^{17} molecules (or 30,000 pmoles of oligonucleotide) at a commercial prize of approximately 300 USD. With 10^{17} molecules, 10^{9} - 10^{11} m^3 can be labelled with 1-100 labels per ml.

LABELLING OF POTENTIAL ENVIRONMENTAL POLLUTANTS

The group of potential environmental pollutants include oil, pesticides, waste chemicals, etc., of which all are chemicals transported in large quantities and which on occasion are illegally or accidentally dumped in the natural ecosystems.

The DNA tags are added to the liquid to a final concentration of a minimum of one molecule per millilitre (2×10^{-9} pM or higher). DNA is not necessarily soluble in all such potential pollutants. This means that the oligonucleotides will make up a dispersion of precipitated DNA rather than DNA in solution, cf. the discussion in the perfume example below. This may, however, be an advantage, since the DNA molecules will be chemically very stable in such a situation. Further, it is not expected that single molecules will sediment over a realistic time-span, but rather stay evenly distributed in the labelled liquid.

An experiment was carried out for testing the method by labelling of (A) commercial gasoline (95 octane unleaded), (B) commercial kerosene and (C) commercial road diesel:

10^8 DNA tags (0.2 pmol 83-mer) dissolved in 10 µl of water was added to 1 ml of A, B and C. The samples were carefully mixed by vortex for 1 min in a 1.5 ml microfuge tube. The 1 ml labelled products A, B and C were further diluted by adding them to 5 l of A, B or C, respectively, and mixed by manual shaking. The new concentration was then 20,000 DNA tags or 3×10^{-9} pmol pr ml.

200 µl samples were removed and centrifuged at 12000 x g for 30 min at +4°C in order to mimic long time (250 days) precipitation at normal gravity (1 x g). Samples of 100 µl (2000 copies), 10 µl (200 copies) and 1 µl (20 copies) were removed together with the empty tube.

The labelled sample (A) was evaporated in a fume hood. Samples (B) and (C) were extracted with 10 mM Tris buffer pH 8 in the presence of 1 µg carrier tRNA, followed by ethanol precipitation, centrifugation at 12000 x g for 10 min at +4°C and drying of the pellet. The dried down non-visible DNAs of (A), (B) and (C) were dissolved in 30 µl distilled water. Detection and deciphering of the encoded message was carried out via 35 cycles of PCR (0.3 µM of each primer, 1.5 u Taq pol, 1,5 mM MgCl2, 0,2 mM dNTP in 0,050 ml reaction volume. PCR: Denaturation at 94°C for 1 min., annealing at 62°C for 1 min, extension at 72°C for 1 min). The result was visualized by gel electrophoresis, staining of DNA with ethidiumbromide and photography in 301 nm UV light and a red filter.

Unfortunately, the above experiment has until now met difficulties due to contamination of the system. There is, however, every reason to believe that detection and characterization with respect to the informational content of between 10^2 - 10^{12} copies of 83-mer will prove possible (as in the example below) when these problems have been solved.

PERFUMES, ALCOHOLIC LIQUIDS

This group of products belongs to a large group of exclusive high cost products which are illegally copied and manufactured as being the original.

The DNA tags are added the liquid to a final concentration of a minimum of one molecule per millilitre (2×10^{-9} pM or higher). DNA is not soluble when the alcohol content is around 70% or more. This means the oligonucleotides will make up a dispersion of precipitated DNA rather than DNA in solution, which is an advantage rather than an disadvantage, since the DNA molecules will be chemically very stable in such a situation. Further, it is not expected that single molecules will sediment over a realistic time-span, but rather stay evenly distributed in the labelled liquid.

The method for demonstration of the highly diluted liquid of DNA tags is polymerase chain reaction (PCR), or any biochemically related enzymatic method. As alternative method, a combination of PCR and DNA sequence analysis is carried out to read the alpha-numeric information of the tag.

An experiment was carried out for testing the method by labelling of 80% alcohol (to mimic perfume). Amounts ranging from 20 pmol to 2×10^{-11} pmol were added to transparent test tubes containing 1 ml of 80% alcohol. The labelled alcohol was stored for 3 days at -20°C. After the incubation period the tubes were shaken and 0.1 ml samples were taken from each concentration and supplied with two matching primers. The samples were lyophilized in 1.5 ml microfuge tubes. The dried down non-visible DNA was dissolved in 30 µl distilled water. Detection and deciphering of the encoded message was carried out via a 30 cycles of PCR (0.3 µM of each primer, 1.5 u Taq pol, 1,5 mM MgCl2, 0,2 mM dNTP in 0,050 ml reaction volume. PCR: Denaturation at 94°C for 1 min., annealing at 58°C for 1 min, extension at 72°C for 1 min). The result was visualized

by gel electrophoresis, staining of DNA with ethidiumbromide and photography in 301 nm UV light.

The result shows that from 0.1 ml of 80% alcohol, 10^2 - 10^{12} copies of a 89-mer could be detected and characterized with respect to sequence and its alphanumeric message with the aid of a PCR reaction. The complete analysis took 3 hours. All sample concentrations gave a positive result with a 89 bp fragment. The lowest concentrations were amplified in two subsequent reactions.

Not even at the highest concentration (10^{12} molecules/ml) any sign of cloudiness in the liquid could be seen.

LABELLING OF PERSONAL ITEMS

This section pertains to labelling which can be used by individuals in order to put their personal label on valuable belongings like antiques, jewellery, furniture, cars, bicycles, cameras, computers etc.

The DNA tags are added to a final concentration of 1,000 - 1,000,000,000 molecules per microliter (2×10^{-3} pM 2,000 pM) to the ink liquid used in permanent pens. DNA is not soluble when the alcohol content is around 70% or more, cf. the above comments.

An experimental description for testing the method is given: 20 pmol of DNA tag is added to 1 ml of transparent permanent ink. The labelled ink is used for filling a permanent pen. The pen is then used for marking (A) a piece of paper and (B) a portable computer. Samples are taken from (A) by soaking the paper in a minimal volume of distilled water and from (B) by carefully scratching the labelled area with a suitable tool. The samples from (A) are lyophilized in 1.5 ml micro-fuge tubes and the dried down non-visible DNA is dissolved in 30 µl distilled water and from (B), the scraped material is introduced into 30 µl of distilled water in order to dissolve

DNA tags. Detection and deciphering of the encoded message is carried out via a 30-40 cycles of PCR (0.3 μM of each primer, 1.5 u Taq pol, 1,5 mM MgCl2, 0,2 mM dNTP in 0,050 ml reaction volume. PCR: Denaturation at 94°C for 1 min., annealing at annealing temperature for 1 min, extension at 72°C for 1 min). The result is visualized by gel electrophoresis, staining of DNA with ethidiumbromide and photography in 301 nm UV light and red filter.

The result is detection and characterization of the DNA tag with respect to sequence content and its alphanumeric message with the aid of a PCR reaction. The complete analysis has a duration of approximately 3 hours.

LABELLING OF PLASTIC PRODUCTS

Plastic products is a large group of plastic products protected by intellectual property rights. However, illegal copies represents a huge economical loss for the companies producing the original product.

Strategies for labelling of LEGO plastic toys: (A) DNA tags are mixed into the dry chemicals before the process of casting takes place or (B) DNA tags are loaded into small protecting spheres before added to the dry chemicals or (C) DNA tags are sprayed onto ready-made LEGO toys.

Alternatively DNA tags also can be added on the package, cover or bar codes etc. of the toys.

Sampling from strategies (A) and (B) involves graining a piece of the toy to a fine powder which is dispersed in a 10 mM Tris buffer pH 8 for dissolving some of the DNA tags. Sampling from strategies (C) is in the simplest situation washing the toy with a small volume of 10 mM Tris buffer pH 8 for dissolving some of the DNA tags, or if necessary, to follow a similar procedure as for (A) and (B).

Detection and deciphering of the encoded message is carried out by 35 cycles of PCR (Example: 0.3 µM of each primer, 1.5 u Taq pol, 1,5 mM MgCl2, 0,2 mM dNTP in 0,050 ml reaction volume. Denaturation at 94°C for 1 min., annealing at 62°C for 1 min, extension at 72°C for 1 min). The result is visualized by gel electrophoresis, staining of DNA with ethidiumbromide and photography in 301 nm UV light and a red filter.

PLACING SERIAL NUMBERS IN MANUFACTURED GOODS

Certain valuable products like cars, bicycles, cameras, computers etc. are valuable even though they are produced in large numbers. However, the identification of the product should normally tie the owner of the product unambiguously to one specific product. Of course, the owner of a car could label the car per own volition, but since cars normally change owners several times, it seems more appropriate to let the car be unambiguously identified "from birth".

The tag may be added to the products either as one single marker for each individual product and/or in the from of a number of patches, each representing a manufacturers general ID with an added number of one or more digits. Thus, a serial number that may or may not be identical to the serial number of the pertinent product may be positioned in one or more places on the product. Since the numbers cannot be read by any third-party, they are virtually impossible to forge.

An example illustrating the use of the method of the invention in this embodiment is given in the following:

20 pmol of DNA tag is added to 1 ml of transparent solvent which is subsequently added to the marking paint or dye. The labelled dye or part is applied to an already painted surface in the order of a serial number.

The reading of the label is performed by carefully scratching the labelled area with a suitable tool. The sample is lyophilized in 1.5 ml microfuge tubes and the scraped materia is introduced in 30 µl of distilled water in order to dissolve DNA tags. Detection and deciphering of the encoded message is carried out via 30-40 cycles of PCR (0.3 µM of each primer, 1.5 u Taq pol, 1,5 mM MgCl2, 0,2 mM dNTP in 0,050 ml reaction volume. Denaturation at 94°C for 1 min., annealing at annealing temperature for 1 min, extension at 72°C for 1 min). The result is visualized by gel electrophoresis, staining of DNA with ethidiumbromide and photography in 301 nm UV light and a red filter.

The result is detection and characterization of the DNA tag with respect to sequence content and its alphanumeric message with the aid of a PCR reaction. The duration of the complete analysis should be about 3 hours.

INTRODUCTION OF CHEMICAL LABELS IN PAINTS AND DYES

Any product which receives an original coating of paint, dye, varnish etc, may be uniquely labelled according to the invention by incorporating a chemical tag in the paint. Because of the trace amounts of the chemical tag it will not have any detectable influence on the colour or lustre of the paint. Further, any trace of the paint will in the future carry the label.

This can be utilized in a number of scenarios:

1. After a hit-and-run car accident, it will often be possible to identify the runaway car by analysis of remnant of car paint found on the scene of the crime. The identification level can range from basic information (brand and type of car, year of production etc.) to specific information (serial number of the car).

2. A stolen car or motorbike can be easily identified or rendered "probably stolen": In the case the stolen car has not been repainted, the labelled paint will provide the authorities with the information outlined above. Alternatively, in the case the car has been repainted, the absence of the chemical label will indicate that the car has been significantly altered.

CHEMICAL LABELLING OF WEAPONS AND OTHER MILITARY EQUIPMENT

Theft of military equipment from military deposits is a quite common problem in *i.a.* the Scandinavian countries and often the stolen goods appear on the illegal market or in connection with other criminal activity. In such cases it is often hard, if not impossible, to trace the origin of the equipment, since all visible or readily available markings, serial numbers etc. have been removed or altered in an attempt to provide the equipment with a new or obscure identity. The invisible marking according to the present invention provides an effective and simple alternative to the identification means already existing. Further, the general knowledge that a high-security labelling system of military equipment exists will probably in itself be an effective bar against theft from military storages, as the risk of being caught will increase.

LABELLING OF BANK NOTES, SECURITIES, BAR CODES ETC.

An ultimate method of detecting of counterfeit bank notes, bonds, letters of credit and other securities is highly desired, since modern technology renders copy protection progressively harder to achieve.

The method of the present invention offers the possibility of incorporating a chemical label in the paper or alternatively in the ink. Since it is possible to incorporate more than one high-security tag in e.g. a bank note it will be possible to

incorporate different tags, each carrying different information (nominal value of the bank note, batch number etc.).

Accordingly, in cases where paper or other products are intended to be bar-coded, the label according to the present invention may be incorporated in the bar-code printing ink.

LABELLING OF ELECTRONIC DEVICES AND OF SOFTWARE

The theft of microprocessors and RAM-circuits (in general known as chips) is at present an increasing problem. The value of such circuitry is by weight higher than that of gold, and the chips reappear in new electronic devices (computers, printers etc.) emerging from a multitude of small illicit manufacturers of electronic equipment. Labelling of chips (e.g. by spraying) according to the invention should enhance the possibilities of a successive outcome of the criminal investigation in such cases.

LABELLING OF ORIGINAL PARTS FOR AIRCRAFTS AND OTHER TRANSPORTATION MEANS

The use of counterfeit parts for aircrafts is a great safety hazard. Since such parts may be prepared from the same materials as the original parts it can be difficult, if not impossible to identify them. For example, insufficiencies of counterfeit parts of metals or alloys are in many cases caused by incorrect heat treatment resulting in inadequate strength and/or resilience. The existence of an invisible label on original parts would increase the awareness of the airlines when they are offered spare parts at a low cost. Further, demonstration of the use of counterfeit parts in an airplane will render possible crippling litigation after an aircraft accident. In other words, the use of an invisible label according to the invention would lead to an increased security in airway travelling and transportation, simply because of the economical implications of neglecting the warranted use of original spare parts.

LABELLING OF ART AND ANTIQUITIES

The present invention offers the possibility of specific labelling works of art or other unique objects. The inventive labelling can be used in this connection in a number of different scenarios:

1. Contemporary artists can authenticate their own works of art by applying an invisible label. The label can e.g. be incorporated in paint or in the canvas (if the work of art is a painting) or in any other suitable material forming part of the work of art. The precise nature of the label can be deposited in an institution which can (at any point in future) test whether a given work of art is in fact authentic.

2. Existing (classical) works of art or antiquities can be labelled by the proprietor, and as in item 1 above, information regarding the precise nature of the label can be deposited in an institution for later verification.

A typical technique would be to spray a DNA tag onto a limited (but undisclosed) part of the labelled object. Later sampling would encompass washing the object with a small volume of 10 mM Tris buffer pH 8 in order to dissolve a portion of the DNA applied. Detection an deciphering: 35 cycles of PCR (Example: 0.3 µM of each primer, 1.5 u Taq pol, 1,5 mM MgCl2, 0,2 mM dNTP in 0,050 ml reaction volume. Denaturation at 94°C for 1 min., annealing at 62°C for 1 min, extension at 72°C for 1 min). The result is visualized by gel electrophoresis, staining of DNA with ethidiumbromide and photography in 301 nm UV light and a red filter.

CLAIMS

1. A method for chemical labelling of an object, the method comprising adding, to the object, at least two chemical tags, a first and a second,

- the first tag comprising an informational content 1) which is not divulged to the public, 2) which can be amplified by use of molecular amplification, and 3) the presence of which specifically establishes the identity and/or origin of the object,

- the second tag comprising an informational content 1) the presence of which indicates that the object is labelled with the first substance, and 2) which is easily detectable.

2. A method according to claim 1, wherein the informational content of the first tag is in the form of an alphanumeric code.

3. A method according to claim 1 or 2, wherein the informational content of the first tag is detectable as a result of the molecular amplification or of a combination of the molecular amplification and sequence analysis.

4. A method according to any of the preceding claims, wherein the first tag comprises a nucleic acid fragment.

5. A method according to claim 4, wherein the informational content of the first tag is comprised in the sequence of the nucleic acid fragment.

6. A method according to claim 4 or 5, wherein the nucleotide sequence of the first tag comprises nucleotides different from A, dA, G, dG, C, dC, U, and T.

7. A method according to claim 7, wherein the nucleotides different from A, dA, G, dG, C, dC, U, and T are selected from the group consisting of

4-acetylcytidine,
5-(carboxyhydroxymethyl)uridine,
2'-O-methylcytidine,
5-carboxymethylaminomethyl-2-thiouridine,
5-carboxymethylaminomethyluridine,
dihydrouridine,
2'-O-methylpseudouridine,
beta,D-galactosylqueuosine,
2'-O-methylguanosine,
inosine,
N6-isopentenyladenosine,
1-methyladenosine,
1-methylpseudouridine,
1-methylguanosine,
1-methylinosine,
2,2-dimethylguanosine,
2-methyladenosine,
2-methylguanosine,
3-methylcytidine,
5-methylcytidine,
N6-methyladenosine,
7-methylguanosine,
5-methylaminomethyluridine,
5-methoxyaminomethyl-2-thiouridine,
beta,D-mannosylqueuosine,
5-methoxycarbonylmethyl-2-thiouridine,
5-methoxycarbonylmethyluridine,
5-methoxyuridine,
2-methylthio-N6-isopentenyladenosine,
N-((9-beta-D-ribofuranosyl-2-methylthiopurine-6-yl)carbamoyl)threonine,
N-((9-beta-D-ribofuranosylpurine-6-yl)N-methylcarbamoyl)-threonine,
uridine-5-oxyacetic acid-methylester,

uridine-5-oxyacetic aid,
wybotoxosine,
pseudouridine,
queuosine,
2-thiocytidine,
5-methyl-2-thiouridine,
2-thiouridine,
4-thiouridine,
5-methyluridine,
N-((9-beta-D-ribofuranosylpurine-6-yl)-carbamoyl)threonine,
2'-O-methyl-5-methyluridine,
2'-O-methyluridine,
wybutosine, and
3-(3-amino-3-carboxypropyl)uridine.

8. A method according to any of the preceding claims, wherein the informational content of the second tag can be amplified by use of molecular amplification.

9. A method according to any of the preceding claims, wherein the informational content of the second tag is in the form of an alphanumeric code.

10. A method according to claim 9, wherein the informational content of the second tag is detectable as a result of the molecular amplification or of a combination of the molecular amplification and sequence analysis.

11. A method according to claim 10 or 11, wherein the second tag comprises a nucleic acid fragment.

12. A method according to claim 11, wherein the informational content of the second tag is comprised in the sequence of the nucleic acid fragment.

13. A method according to any of claims 4 to 12, wherein the molecular amplification is in the form of a polymerase chain reaction (PCR) or a PCR combined with a sequence analysis.

14. A method according to any of claims 4-13, wherein the first and second tags are part of the same molecule.

15. A method according to claim 14, wherein the second tag is in the form of nucleotide fragments which flank the nucleotide fragment comprised in the first tag.

16. A method according to claim 15, wherein the nucleotide fragments making up the second tag serves as templates for primers in a PCR reaction which amplifies the informational content of the first tag.

17. A method according to any of claims 1-7, wherein the second tag is selected from the group consisting of a nucleotide fragment, a rare metal, a substance capable of forming micelles, a stain, a fluorescent dye, a specific binding partner such as an antigen capable of binding to an antibody coupled to a marker designed for easy detection or an antibody capable of binding to an antigen coupled to a marker designed for easy detection, and an enzyme capable of catalyzing an easily detectable chemical reaction.

18. A method according to any of claims 1-13 and 17, wherein the first tag and the second tag are not part of the same molecule.

19. A method according to any of the preceding claims, wherein the first and/or second tag is formulated so as to ensure compatibility with the object or wherein a moiety forming part of the first and/or second tag ensures compatibility with the object.

20. A method according to claim 19, wherein the moiety of the first and/or second tag is covalently or non-covalently attached to the remainder of the tag.

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21. A method according to claim 19, wherein the first and/or second tag is formulated together with a micelle forming substance or a detergent.

22. A method according to any of the preceding claims, wherein the second tag is in the form of a defined standard.

23. A method according to any of the preceding claims, wherein the object is a non-living article, product or composition.

24. A method according to claim 25, wherein the non-living article, product or composition is selected from the group consisting of an industrial product, a work of art, an antiquity, an environmental pollutant, air pollutants, oils and petrochemical products, aromatic and aliphatic compounds, explosives, foods and feeds, medicaments, inks, paper goods including securities such as bank notes and bonds.

25. A method according to any of claims 1-22, wherein the object is a living organism or a virus.

26. A method according to claim 25, wherein the living organism is selected from the group consisting of a prokaryotic organism selected from the group consisting of a bacterium, a blue green algae, and an intracellular parasite such as a mycoplasma; an eukaryotic organism selected from the group consisting of a yeast, a fungus, a protozoa, an algae, and a multicellular plant or animal; and cells/tissues derived from a multicellular plant or animal.

27. A method according to any of the preceding claims, wherein the informational content of the first tag is assigned specifically to one single object.

28. A method according to any of claims 1-26, wherein the informational content of nucleotide sequence is assigned specifically to a group of objects.

29. A method according to any of the preceding claims, wherein the first tag is subject to modification and/or loss of at least a part of the informational content as a consequence of its exposure to a physical or chemical condition.

30. A method according to any of the preceding claims, wherein the first and/or second chemical tag is added to the object by being suspended in the object, covalently or non-covalently bound to the object or subunits of the object, or applied as a film or a spray on the object.

31. An object comprising a chemical label which contains

- a first chemical tag comprising an informational content 1) which is not divulged to the public, 2) which can be amplified by use of molecular amplification, and 3) the presence of which specifically establishes the identity and/or origin of the object,

- a second tag comprising an informational content 1) the presence of which indicates that the object is labelled with the first substance, and 2) which is easily detectable.

32. An object according to claim 31, wherein the first and second chemical tags are as defined in any of claims 2-29.

33. A method of determining the identity and/or origin of an object, the method comprising subjecting an object labelled by a method according to any of claims 1-30 or an object according to claim 31 or 32 to a determination which involves the steps of

- detecting the presence and/or extracting the informational content of the second tag, thereby obtaining an indication that the object is labelled with a first tag as defined in claim 1, and subsequently

- detecting the presence and/or extracting the informational content of the first tag by methods involving molecular amplification of the first tag or molecular amplification in combination with sequence analysis of the first tag.

1/1

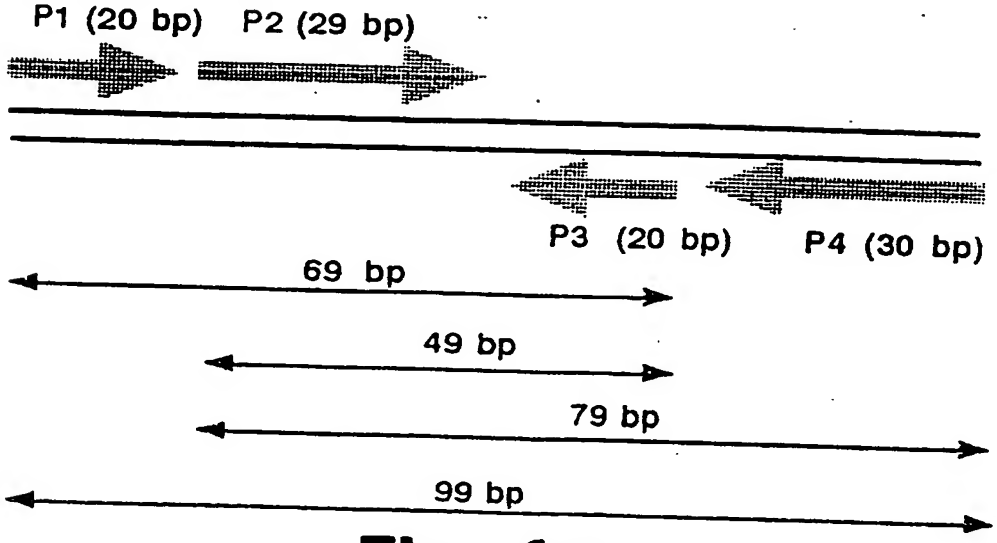

Fig. 1a

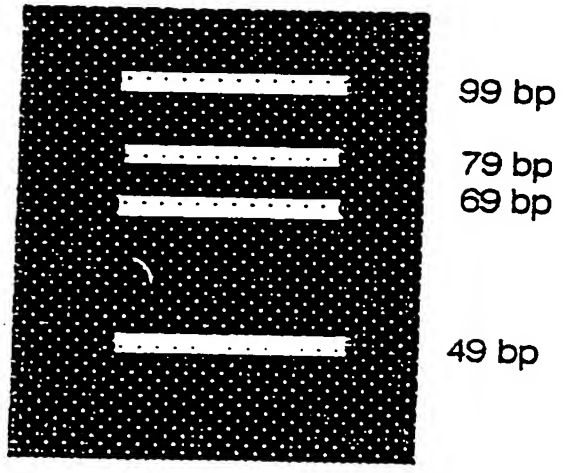

Fig. 1b

INTERNATIONAL SEARCH REPORT

Inter nal Application No
PC1/IB 95/01144

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12Q1/68 G09F3/00 D21H21/46 G01N33/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO,A,94 04918 (SLATER JAMES HOWARD ;MINTON JOHN EDWARD (GB)) 3 March 1994 cited in the application see the whole document ---	1-33
X	WO,A,94 16902 (BUTLAND TRUST ORGANIZATION) 4 August 1994 see the whole document ---	1-5, 31-33
A	WO,A,90 14441 (CETUS CORP) 29 November 1990 cited in the application ---	
A	EP,A,0 408 424 (BIOPROBE SYSTEMS) 16 January 1991 cited in the application ---	
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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
18 April 1996	02. 05. 96

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PC:/IB 95/01144

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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A	WO,A,87 06383 (BIOTECHNICA LTD) 22 October 1987	
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INTERNATIONAL SEARCH REPORT

Information on patent family members

Inter onal Application No
PC1/IB 95/01144

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		EP-A-	0657028	14-06-95
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